UNIVERSITY OF HOHENHEIM

Institute of Plant Breeding, Seed Science and Population Genetics

Chair of Applied Genetics and Plant Breeding

Prof. Dr. A. E. Melchinger • PD Dr. J. M. Montes

Characterization and management of *Jatropha curcas* L. germplasm

Dissertation presented by Elisa Senger

submitted to the Faculty of Agricultural Sciences

Stuttgart - Hohenheim

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Abbreviations

EU	European Union
GxE	Genotype by environment interaction
GMO	Genetically modified organism
HPLC	High-performance liquid chromatography
Jatropha	*Jatropha curcas* L.
PE	Phorbol esters

I General introduction

Jatropha curcas L. (jatropha) is a perennial plant of the Euphorbiaceae family that grows in the tropics and subtropics (Contran *et al.*, 2013) and has a high potential to be exploited as crop species. The seeds of jatropha have a high content of oil that is appropriate for conversion into biodiesel (Ashraful *et al.*, 2015). Therefore, it has received great attention as bioenergy plant in recent years. Food or feed are further attractive products of jatropha plantations (Vera-Castillo *et al.*, 2014). Striking characteristics of this stem-succulent plant are its ability to survive drought periods and to recover degraded soils during cultivation (Wani *et al.*, 2012). For these reasons, jatropha is targeted to be cultivated in marginal environments, *i.e.*, land that is unsuitable for cultivation of row crops (Quinn *et al.*, 2015). Besides sustainable production of bioenergy or food, jatropha cultivation has important environmental benefits such as greening of wastelands, carbon sequestration, and erosion control, amongst others (Pandey *et al.*, 2012; Becker *et al.*, 2013). It is assumed that it originates from Mexico and northern Central America because in these regions, the highest genetic diversity was found (Pecina-Quintero *et al.*, 2014). Domestication of jatropha took place in ancient times in Mexico (Dias *et al.*, 2012; Sanou *et al.*, 2015). Nowadays, jatropha occurs worldwide because it was spread by Portuguese seafarers in colonial times (Montes-Osorio *et al.*, 2014) and it is mainly used as living fence, medicinal plant or for hygienic purposes by the local population (Jiofack *et al.*, 2010; Yongabi *et al.*, 2011). Jatropha is grown for food production only in the South of Mexico (from the states Michoacán and Guerrero in the West to Yucatán and Quintana Roo in the East). Here, local inhabitants that originate from the cultural group of Totonacas cultivate it in their house gardens since ancient times (Valdes-Rodriguez *et al.*, 2013).

Germplasm pools defined by the presence of phorbol esters

Jatropha naturally contains chemical compounds called phorbol esters (PE). PE are diterpenes of the tigliane type with tumor-promoting, inflammatory and other negative effects on humans and animals (Sabandar *et al.*, 2013). PE cannot be removed by heat treatments and

other detoxification approaches have not yet been proven on an industrial scale (JatroSolutions GmbH, personal communication, 2016). Thus, to exploit jatropha as a food or feed crop, cultivars lacking PE shall be developed by plant breeders. Jatropha germplasm can be separated into two naturally occurring germplasm pools that differ in the presence of PE: One pool contains PE (toxic germplasm pool), the other pool does not (non-toxic germplasm pool). King *et al.* (2013) investigated the inheritance of PE and found that the PE absence of the naturally occurring non-toxic accessions is a monogenic, maternally controlled, recessive trait. According to the authors, PE biosynthesis takes place in maternal tissues of toxic plants. Within the seeds, PE are concentrated in the tegmen and diffuse into the endosperm due to their lipophilic character. Within the toxic pool, phorbol ester content in seeds can vary among genotypes. Thus, when phorbol esters are present, the content in seeds is a quantitative trait (King *et al.*, 2013). Furthermore, different molecular markers associated to the presence of PE were identified in their study that can be exploited in breeding programs or for the development of food safety strategies.

Six different PE compounds were identified in jatropha and called "jatropha factors 1 - 6", all of which possess the same diterpene unit 12-deoxy-16-hydroxy-phorbol. All six factors seem to be unstable molecules. They are generated by intramolecular reactions or rearrangements while maintaining the basic diterpene structure (Baldini *et al.*, 2014). Terpenes are a large family of carbohydrates that are synthesized from several units of isoprene. Vranova *et al.* (2012) described the terpenoid pathways in plants and highlighted that diterpenes are synthesized from geranylgeranyl diphosphate either in the cytoplasm or in the chloroplasts. Nakano *et al.* (2012) elucidated PE biosynthesis in jatropha and found only one casbene synthase gene ("*Jatropha curcas* casbene synthase homolog", *JcCSH*), which is active in chloroplasts and expressed in seedlings, leaves, and fruits, but inactive in seeds. However, the authors did not reject the hypothesis that there might be other casbene synthase genes that were not detected during their study. Li *et al.* (2016) isolated two casbene synthase genes, namely *JcCASD163* and *JcCASD168* from jatropha. According to the authors, the first one was nearly identical to *JcCSH* isolated by Nakano *et al.* (2012) and active in many plant

tissues, whereas *JcCASD168* was seed specific and particularly expressed in the tegmen of the seed. Both genes showed to have high activity during seed maturation (Li *et al.*, 2016).

Interestingly, gibberellic acid, a phytohormone that promotes cell elongation and internode distance, is a diterpene as well (Vranova *et al.*, 2012). Non-toxic jatropha plants were reported to have a "loose inflorescence", *i.e.* elongated peduncles, and oval fruits (Mulpuri *et al.*, 2013), which might hint on a high level of gibberellic acid present during inflorescence formation and fruit ripening. Non-toxic jatropha plants, which do not synthesize PE, might channel the surplus of the PE precursors geranylgeranyl diphosphate or casbene into the synthesis of other compounds.

Range of jatropha products

The toxic germplasm pool of jatropha was under the spotlight of researchers, investors, and farmers for sustainable production of bioenergy in recent years. The main product of a plantation of toxic jatropha is oil, which is refined and processed to obtain biofuel. As byproducts, fuel pellets can be produced from fruit husks and seed shells, and organic fertilizer from the oil extraction residues (Figure 1). Additional options such as production of industrial raw materials or greening of wastelands are considered (JatroSolutions GmbH, personal communication, 2016; Figure 1). Furthermore, insecticidal and molluscicidal effects of phorbol esters were observed (Sabandar *et al.*, 2013). Thus, jatropha extracts could also be exploited for plant protection. Summarizing, different products or product combinations can be obtained from a jatropha plantation (Figure 1). However, most of them are still to be investigated.

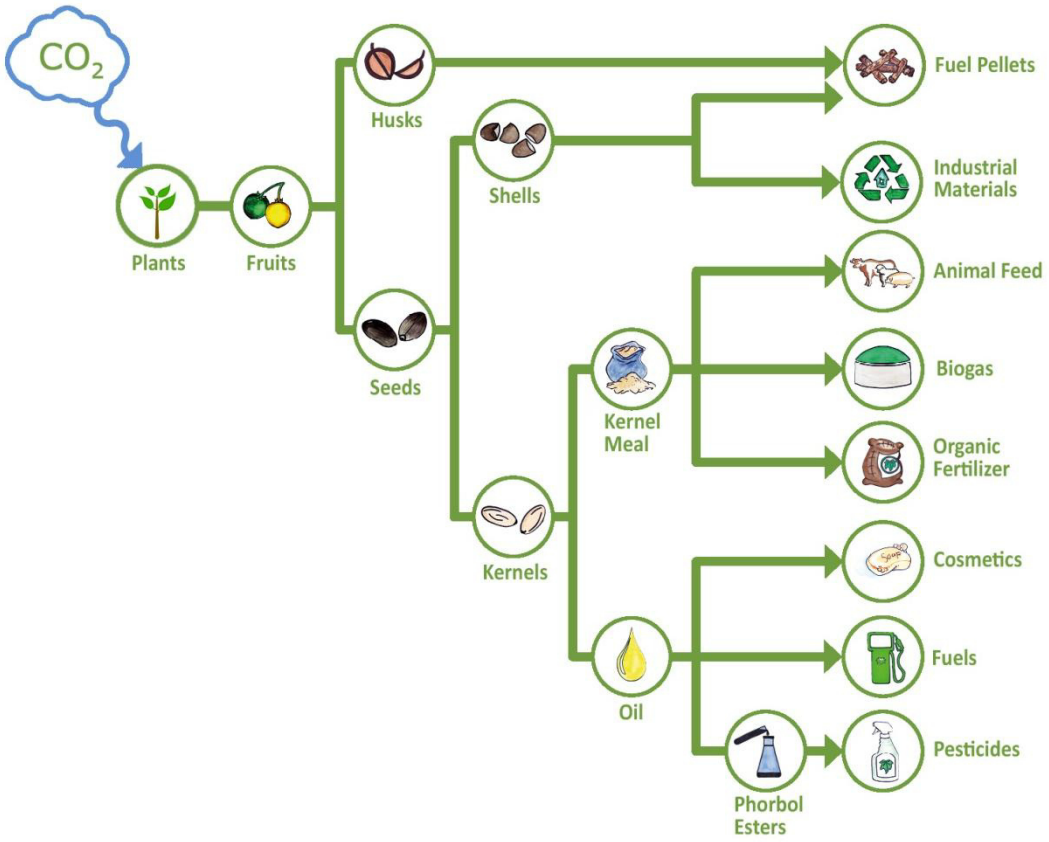

Figure 1 Product options for the toxic germplasm pool (© JatroSolutions GmbH)

The other germplasm pool lacking PE is explored for production of food or feed (Figure 2). If vegetable oil is produced, the remaining press cake of kernels can be used as source of vegetable protein or to produce kernel meal. Alternatively, kernels can be roasted entirely and used as snack (which is one of the traditional uses in Mexico) or as ingredient in *e.g.* bakery products or energy bars. Kernels or kernel fragments can be processed to oil rich kernel meal for bakeries or used as basic material for meat substitutes. Byproducts can be channeled into production of bioenergy or organic fertilizer (Figure 2). Food production from jatropha is currently intensively investigated and needs further improvement mainly in the areas of post-harvest treatments and processing before it can be realized on an industrial scale (Senger *et al.*, 2017).

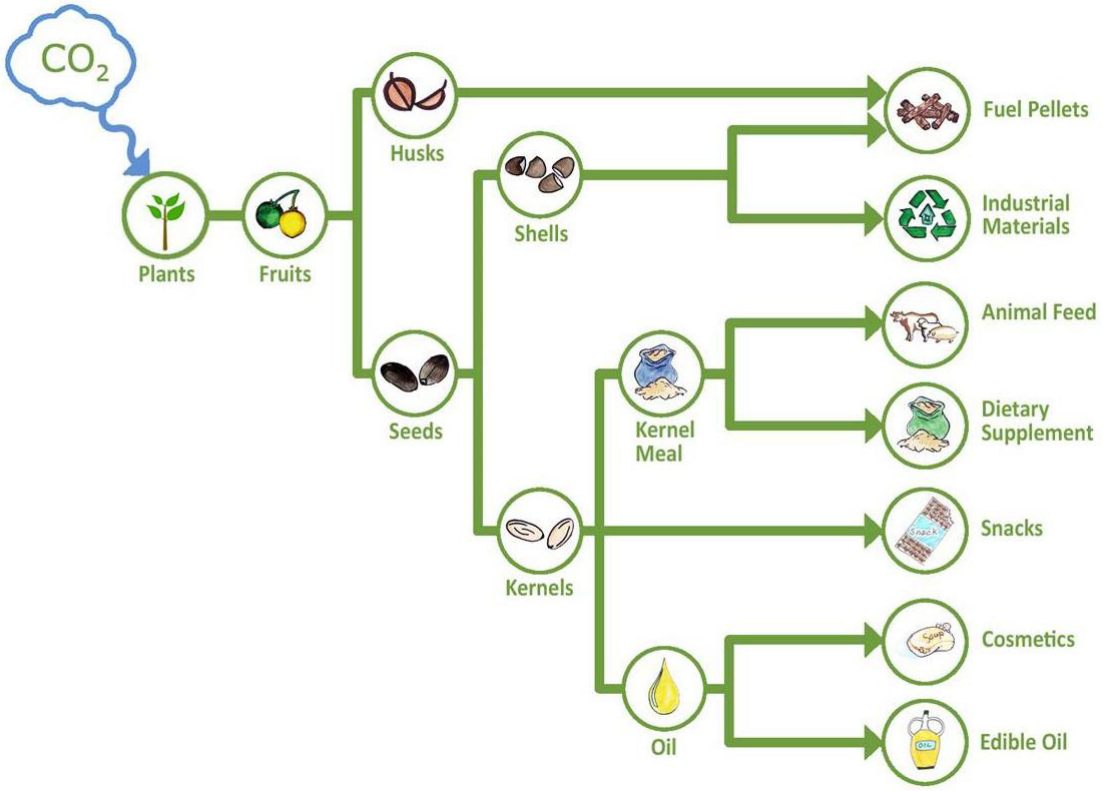

Figure 2 Product options for the edible germplasm pool (© JatroSolutions GmbH)

Genetic and phenotypic diversity

Genetic diversity forms the basis for selection and therefore for every breeding program. In jatropha, high genetic diversity was found: Montes *et al.* (2014) analyzed 70 jatropha accessions with SSR and SNP markers and found a higher genetic diversity in the germplasm pool that lacks PE than in the other germplasm pool. Recent studies confirm high genetic diversity, especially among accessions from Mexico and Central America (Montes-Osorio *et al.*, 2014; Outtara *et al.*, 2014; Santos *et al.*, 2016). Among phenotypic traits, high diversity was found in plant morphology and yield components (Martin and Montes, 2014; Aguilera-Cauich *et al.*, 2015), as well as product quality traits such as PE content (He *et al.*, 2011; Montes *et al.*, 2013; Senger *et al.*, 2017). Most studies that investigated genetic diversity and perspectives for breeding programs came to the same conclusion: There exist high genetic diversity and huge potential to increase seed and oil yield in jatropha.

From the breeders' point of view, monitoring the germplasm across different environments is part of the germplasm characterization. Trait expression is influenced by the plants' genetics (G), the environment (E) and the interaction between them (GxE). For precise selection, breeders need to consider all three factors. Especially GxE effects need to receive special attention to identify genotypes with constant performance across different environments. This reduces the risk of unexpected behavior of the plants in environments that were not included within the range of testing sites. Multi-environmental field trials have recently been set up and detected significant GxE effects on various traits in jatropha germplasm (Tar *et al.*, 2011; Martin and Montes, 2014; Rao *et al.*, 2015; Senger *et al.*, 2016). After intensive field testing, genotypes with highest yield performance, yield stability and other relevant traits can be selected to be released as cultivars or to serve as parental genotypes for subsequent breeding cycles.

Breeding techniques available in jatropha

Generating new cultivars by trait recombination relies on controlled crosses of parental genotypes. This implies the application of a technique to eliminate or exclude the pollen of the maternal plant and a technique to transfer the pollen from the paternal to the maternal plant. Development of these techniques calls for investigation of the flowering behavior and natural mating system of the plant species. Jatropha is a monoecious, insect pollinated plant and the flowers are arranged in inflorescences classified as compound cymes with several dichasia (Noor Camellia *et al.*, 2012). One plant forms several inflorescences during the growing season at its branch tips. Flowering is induced when minimum requirements in temperature, light intensity and water availability are reached. Thus, flushes occur one or several times during the growing season (Noor Camellia *et al.*, 2012).

Within one inflorescence, few female flowers and many male flowers are formed (Noor Camellia *et al.*, 2012). There are visible differences between the male and female flower buds (Dasumiati *et al.*, 2015). Male flowers can thus be removed from the inflorescence prior to anthesis by emasculation with tweezers. However, this is possible in small scale only due to

high demand of manpower. Naturally occurring mutants were found that do not form male flowers. These are called “female-type” (Nietsche *et al.*, 2013), “pistillate” (Palupi *et al.*, 2014), or “male sterile” (Argollo-Marques *et al.*, 2013). Utilization of this trait is important for large scale hybrid seed production. To guarantee isolation of emasculated and pollinated inflorescences, a net, a bag or spacial distance to other plants has to be implemented. It is known that jatropha is self-compatible (Palupi *et al.*, 2014). Thus, selfings can be performed by isolating an inflorescence or plant of a monoecious genotype prior to anthesis.

However, manual emasculation and pollination operations are not applicable for efficient large scale seed production due to the high demand of manpower. Alternatively to seeds, cultivars might be multiplied vegetatively. Protocols are available for production of stem cuttings (Severino *et al.*, 2011), micro cuttings (Pimenta *et al.*, 2014), or *in vitro* clonal propagation (Gangwar *et al.*, 2016). Anyway, the option to multiply a cultivar by seeds has several advantages over vegetative multiplication. For instance, comparing seeds to stem cuttings, multiplication rate is manifold higher, storability is prolonged, transfer across country boundaries is easier, and spread of pathogens that grow in vegetative plant organs is prevented. On the other hand, highest genetic purity is guaranteed by cuttings.

Molecular markers are an important supporting tool of modern breeding. For germplasm characterization, an array of markers can be used. For efficient selection on specific traits, a set of few markers can be used in a marker-assisted selection scheme (Sujatha, 2013). Combination with traditional breeding techniques will accelerate breeding progress to a high degree and accelerate the release of improved cultivars. For instance, molecular markers associated to male sterility or early flowering would highly increase breeding efficiency for these traits because they can be monitored for the first time during flowering, a rather advanced developmental stage of the plant. Information on molecular markers available for jatropha is scarce. King *et al.* (2013) identified a locus that controls the biosynthesis of phorbol esters and published several markers that are linked to it. Information on other markers linked to specific crucial traits will soon become available due to the growing interest in jatropha.

Jatropha breeding

Genetically improved cultivars are needed to increase the profitability of jatropha farming (Yue *et al.*, 2013). The main breeding objective is currently to increase seed yield as well as its stability across years and environments. For this challenge, multi-locational field trials to evaluate the crop performance across different environments and several years were set up recently by independent working groups, *e.g.*, in Brazil (Laviola *et al.*, 2013), India (Chikara *et al.*, 2013), Malaysia (Biabani *et al.*, 2012), Thailand (Tar *et al.*, 2011), and globally (Martin and Montes, 2014). Senger *et al.* (2016) found high GxE interactions in jatropha and concluded that multi-location field trials are needed to select genotypes with high and stable yield. Further breeding objectives depend on the target market of jatropha products. Oil yield and oil quality parameters are improved for biofuels, whereas for food products, oil or protein yield and contents of secondary plant metabolites are crucial. In the case of jatropha, knowledge on optimal agronomic management practices is lacking (Singh *et al.*, 2013). Thus, besides yield components, also traits of agronomic interest are under the spotlight of breeders. Independent from the target market, fast early development of juvenile plants, high water and nutrient efficiency, tolerances and resistances to abiotic and biotic stresses are required. The plant architecture is crucial for optimal spacing and pruning. Especially for development of a mechanized harvest technique, dwarfs or cultivars with reduced plant height and canopy spread are needed, in particular in the biofuel segment due to the low product price.

Jatropha breeding is at an early stage. Mainly wild jatropha accessions collected in gene banks or present in natural habitats around the world represent the germplasm. The few existing jatropha breeding groups were founded in recent years and are currently focusing on characterization and evaluation of their germplasm, investigation of agronomic management practices, development of breeding techniques, and optimization of processing technologies. Few first generation cultivars were already commercialized by different breeding groups (Hawkins and Chen, 2015). Crucial traits of interest that are monitored during this initial phase have to be defined according to the breeding objectives. However, not all traits can be assessed at the same time and with equal intensity. Setting wise priorities and applying selection indices is advisable. Furthermore, different methods for data assessment have to be

compared. For instance, traits that are difficult to measure shall be replaced by traits that are easier to assess but that are highly associated to the trait of interest, *e.g.* kernel weight can be replaced by seed weight due to a high correlation (Montes *et al.*, 2013). In this case, manpower and therefore costs are reduced because cracking of seeds is avoided. Similarly, measuring SPAD value instead of chlorophyll content or nitrogen efficiency (Senger *et al.*, 2014) saves manpower as well as laboratory equipment and materials. Furthermore, associations of crucial traits of interest need to be identified and taken into account for decision on the further selection strategy because negatively associated traits cannot be improved at the same time, such as oil and protein contents in kernels (Montes *et al.*, 2013).

Having identified PE to be a crucial trait with regard to the choice of the target market of jatropha products, dividing the germplasm into at least two germplasm pools that differ in presence of PE is recommendable to breeders. Due to the importance of this trait for assigning cultivars to a specific market, clear differentiation is crucial to prevent any contamination of the germplasm pool that lacks PE. Molecular markers are available to identify PE synthesizing plants (King *et al.*, 2013). However, alternative methods need to be developed, too, for instance for sample material such as oil, where no DNA is present for molecular analyses. Here, the HPLC approach developed by Devappa *et al.* (2010) is most promising to be applied as a standard method for detection of PE in the food industry. To facilitate germplasm management in the breeding program, phenotypic markers that are linked to the PE presence would save costs and time and therefore enhance breeding efficiency. To my best knowledge, no studies reporting phenotypic markers that are linked to PE presence in jatropha are published. However, promising preliminary studies do exist (Montes *et al.*, 2015; Senger *et al.*, 2015).

Objectives

The objectives of this study were to

i. examine the variation of relevant traits among genotypes and between germplasm pools,
ii. estimate phenotypic and genotypic trait correlations,
iii. investigate location effects and genotype by environment interactions,
iv. investigate parental and heterotic effects of genotypes from different germplasm pools as well as the effect of the mating type on expression of relevant traits,
v. develop recommendations for implementation of the findings in jatropha breeding programs.

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II Genetic variation in leaf chlorophyll content of *Jatropha curcas* L.

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Industrial Crops and Products, 2014 58:204-211

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Abstract

Jatropha curcas L. (jatropha) has recently received great attention for its utilization in biofuel production, rehabilitation of degraded land and rural development. Knowledge of plant physiology is important for breeding and agronomy. Photosynthesis is one of the key physiological processes on Earth. We investigated the genetic variation in leaf chlorophyll content of jatropha. Using a high-throughput methodology, 257 accessions were screened in the field. Measurements were performed at three locations repeatedly during the vegetation period. Genetic variation of this trait was large among the genotypes tested showing a high potential for adaptation to changing environments. We found genotypes with consistent high level and low level of leaf chlorophyll content as well as genotypes that showed cross-over interactions across environments. Leaf chlorophyll content was associated with flowering level, but only weakly with seed mass. Measurements of leaf chlorophyll content were associated with abiotic stress. We conclude that leaf chlorophyll content is a viable trait to assess the physiological status of a plant in the field during the vegetation period. It might serve for the detection of stress symptoms, but agronomic applications have to be calibrated for each specific genotype.

III Laboratory screening of aluminum tolerance in *Jatropha curcas* L.

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Industrial Crops and Products, 2014 59:248-251

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Abstract

Jatropha curcas L. (jatropha) has recently received great attention for its utilization in biofuel production and rehabilitation of degraded land. Jatropha grows in tropical and subtropical regions where large extensions of acidic soils with aluminum contamination are present. It is therefore important to screen the tolerance of jatropha accessions to this metal. We assessed aluminum tolerance levels in eight genotypes by means of a laboratory seed germination/root elongation toxicity test. The plant material was exposed to solutions of different aluminum concentrations in sand susbtrate. Days to germination, root length, shoot length, shoot dry matter, and leaf chlorophyll content were measured. Aluminum exposure of plant material significantly influenced the days to germination, root length and leaf chlorophyll content. The aluminum influence on these three traits was combined in an index, which revealed genetic differences among the tested genotypes. Potential aluminum tolerance was identified in G8, whereas G1 and G5 showed the highest aluminum sensitivity. However, the level of aluminum tolerance identified in the laboratory needs to be confirmed in the field.

IV Quantitative trait variation in self- and cross-fertilized seeds of *Jatropha curcas* L.: Parental effects of genotypes and genetic pools

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Abstract

Jatropha curcas L. (jatropha) is an undomesticated plant with a high potential for sustainable production of biofuels in the tropics and subtropics. Oil content, seed mass, kernel mass, and the ratio of kernel to seed mass are target traits for breeding. These traits could potentially be improved by exploitation of parental effects. Additionally, parental effects could be used to separate self- from cross-fertilized seeds and increase the efficiency of hybrid seed production. The main goal of this study was to assess the variation of quantitative seed traits (oil content, seed mass, kernel mass, and ratio of kernel to seed mass) in a panel of self- and cross-fertilized seeds from a genetically diverse germplasm. We analyzed 31 genotypes (12 self- and 19 cross-fertilized genotypes) originated by combination of parental components from 2 different genetic pools (pool A: phorbol esters absent; pool B: phorbol esters present). We found significant effects of parental genotypes and genetic pools on most seeds traits. Although the variation in oil content among single seeds was very large, some specific combinations of parental genotypes showed promise to be exploited for rapid and automated identification and classification of self- and cross-fertilized seeds. We conclude that parental effects need to be considered for breeding and cultivar seed production.

V Classification of *Jatropha curcas* L. genotypes into germplasm groups associated with the presence of phorbol esters by means of seed characteristics

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Industrial Crops and Products, 2015 78:9-12

DOI: 10.1016/j.indcrop.2015.10.005

Abstract

Jatropha curcas L. (jatropha) is a perennial plant with a high potential for sustainable production of food, feed and biofuels in the tropics and subtropics. Breeding has recently been initiated. Easily assessable seed traits would be useful for germplasm classification and management. The main goal of this study was to investigate the potential of seed traits to classify genotypes into germplasm groups associated with the presence of phorbol esters. The seed traits were evaluated in a panel of 31 genotypes (12 self-fertilized and 19 cross-fertilized). Within the self-fertilized genotypes, all traits showed clear contrasting frequencies between the germplasm groups. Within the cross-fertilized seeds, paternal effects were present for specific genotype combinations. We conclude that classification of genotypes into germplasm groups associated with the presence of phorbol esters by means of seed traits is promising but needs further investigation with a larger set of genotypes, plants per genotype and environments. These results are of importance for management and characterization of jatropha germplasm.

Senger E, Martin M, Montes JM (2015) Ind Crop Prod 78:9-12

VI Parental and heterotic effects in *Jatropha curcas* L. seedlings

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Tropical Plant Biology, 2016 9(1):42-49

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Abstract

Jatropha curcas L. (jatropha) is an important undomesticated perennial plant with high potential for sustainable production of food and biofuel in the tropics and subtropics. However, jatropha has no breeding history and genetic improvement of this novel crop has just been initiated recently. Therefore, it is of utmost importance to understand the significance of parental and heterotic effects that could be exploited in hybrid combinations. The main goal of this study was to assess the parental and heterotic effects on seedlings. Our objectives were to (i) examine the variation of traits among genotypes, between genetic pools and mating types, (ii) investigate the parental and heterotic effects on these traits, and (iii) discuss the exploitation of parental and heterotic effects in jatropha breeding. Genotypes from two genetic pools were used to perform intra- and interpool crosses. Data on germination time, plant height, area of cotyledon and primary leaf, chlorophyll content of primary leaf, number of leaves, and shoot dry mass were recorded. According to our results, maternal and paternal effects affected the expression of seedling traits in jatropha. The level of influence depended on the trait and the parents under consideration. Heterotic effects were present for all seedling traits. The largest heterotic effect was found in an interpool cross.

VII Genetic variation and genotype by environment interaction in *Jatropha curcas* L. germplasm evaluated in different environments of Cameroon

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Abstract

Jatropha curcas L. (jatropha) is a perennial plant with a high untapped potential towards sustainable production of food and bioenergy. The transformation of jatropha into a competitive crop requires intensive breeding efforts. The objectives of our study were to (i) assess genetic variation of agronomic and quality traits in different environments, (ii) investigate genotype by environment interactions, and (iii) discuss potential selection strategies. Agronomic and quality traits were assessed on 277 jatropha accessions that were evaluated over three environments in Cameroon. Genetic variation and heritability of agronomic and quality traits showed excellent prospects to select and breed improved cultivars rapidly. Selection for accumulated seed yield over years seems to be the best choice to achieve high seed and oil yield levels. Seed yield per month might be incorporated in selection indices to improve the harvest efficiency. Selection based on a single testing environment was always inferior to the selection based on multiple environments. The magnitude of genotype by environment interaction (GxE) in jatropha is large. Therefore, testing in multiple environments is a requirement to select improved cultivars with local and broad adaptation.

Senger E, Martin M, Dongmeza E, Montes JM (2016) Biomass Bioenerg 91:10-16

VIII Chuta (edible *Jatropha curcas* L.), the newcomer among underutilized crops: A rich source of vegetable oil and protein for human consumption

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European Food Research and Technology, 2017 243(6):987-997

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Abstract

Facing the worldwide increasing demand for edible oil and protein and their production deficit in many developing countries, we investigated the nutritional value of chuta (edible *Jatropha curcas* L.). Chuta is a perennial tropical and subtropical plant that survives in unfavorable environments. Chuta cultivation requires less input than cultivation of common cash crops. The oil- and protein-rich kernels can be exploited as a snack, as an ingredient for foodstuffs, or for production of edible vegetable oil and protein. We analyzed seed and kernel characteristics, the fatty and amino acid profiles, vitamin and vitamer contents, and the levels of minerals in kernels of 40 chuta genotypes and compared mean values to plant species commonly used as vegetable oil and protein sources. Our results showed that chuta oil and protein have high nutritional value for humans. We concluded that chuta products can compete with products of other crops such as soybean and peanut. Chuta products can complement the diets of vegetarians and vegans, professional athletes or persons who have to restrict their consumption of carbohydrates for medical reasons. Further, chuta can be cultivated in rural areas of developing countries, where protein sources might be scarce. Some parameters investigated here can be influenced by environmental factors during plant growth and factors of the processing chain. Considering the excellent nutritional value and promising breeding opportunities to improve important parameters, we expect an expansion of the cultivation area of this underutilized crop in the near future for production of edible vegetable oil and protein.

Senger E, Bohlinger B, Esgaib S, Hernández-Cubero LC, Montes JM, Becker K (2017)
Eur Food Res Technol 243(6):987-997

IX General discussion

Breeding objectives in jatropha

The lack of competitive cultivars for feasible jatropha cultivation led to initiation of breeding programs in recent years. The optimal breeding category is not yet defined, but hybrid breeding seems promising (Montes and Melchinger, 2016). In the current phase of jatropha breeding, setting of breeding objectives and priorities along with optimal resource allocation is of utmost importance to achieve high selection gains. The primary breeding objectives for any crop are to increase yield and yield stability. Further, reduction of the production costs and improved product quality are targeted. Germplasm screenings are needed to assess the genetic diversity and heritability in relevant traits and to evaluate the perspectives for genetic improvement.

For the production of bioenergy or food from jatropha, oil yield and/or kernel yield of plantations need to be increased. Montes *et al.* (2013) and Martin and Montes (2014) found high correlations between single seed mass and single kernel mass as well as between oil yield and seed yield per hectare. Thus, the primary breeding objective can be summarized for both market segments to increase seed yield per hectare. Since jatropha is targeted to be cultivated in marginal regions, yield stability is particularly important to reduce the risk of yield losses due to unexpected climate events or similar incidents.

Quality traits have to be considered in breeding programs. Depending on the product, different quality traits are relevant. Thus, breeding cannot be performed simultaneously for all products. In the case of jatropha, the most promising markets are bioenergy and food. High diversity in quality traits of jatropha was found (Montes *et al.*, 2013; Yi *et al.*, 2014) that can be exploited for improvement of the product quality or for the development of novel products. However, environmental factors were proven to affect quality traits (Pompelli *et al.*, 2010; Montes *et al.*, 2014; Senger *et al.*, 2017) and need to be considered in jatropha cultivation.

At the current stage, it is arguable if breeding for specific markets is advisable. Any effort undertaken by the breeder needs to be rewarded by the seed market. For instance, the

release of sunflower and canola cultivars with specific fatty acid composition was justified by an increasing demand for bioenergy and the large cultivation area of these crops. In 2014, canola was grown on a total area of approx. 36 Mio. ha, sunflower on approx. 25 Mio. ha (FAO, 2017). A study summarizing and comparing jatropha projects worldwide estimated that a total area of approx. 1.2 Mio. ha was planted with jatropha in 2012 (Wahl *et al.*, 2012). The first obstacle in jatropha breeding is to elevate yield levels for profitable cultivation. As soon as the cultivation area is growing, focusing on quality traits or release of cultivars adapted to specific environments will be justified by larger seed markets.

Characterization of jatropha germplasm

The first prerequisite for directed breeding is to characterize the available material in traits that are relevant for the specific breeding objectives. Efficient methods need to be implemented for systematic data collection. Since the major breeding objective in jatropha is to increase seed yield and yield stability, the understanding of how yields are generated is of crucial importance. Yield components and flowering traits have been examined in detail and high heritabilities have been found for accumulated seed yield, hundred seed mass and flowering duration (Shabanimofrad *et al.*, 2013; Tripathi *et al.*, 2013; Martin and Montes, 2014; Arolu *et al.*, 2015). However, multi-factoral studies on yield stability, *i.e.*, environmental adaptation, including a diverse set of genotypes tested in different environments over several years are scarce. In particular flowering traits can be highly influenced by abiotic and biotic stress factors (Noor Camellia *et al.*, 2012; Domiciano *et al.*, 2014). Considering the potential target sites for jatropha cultivation in the tropics and subtropics, most important abiotic stresses are drought (either low precipitation or short vegetation period) and nutrient deficiencies caused by poor soil fertility and by low-input cultivation practices commonly applied by smallholders with low purchasing power.

The performance of germplasm has to be tested in the potential target sites of crop cultivation. Superior genotypes with high yield stability, *i.e.*, genotypes showing low GxE interactions, are selected from long-term field trials that reflect the range of possible

prevailing environmental conditions. In a study comparing the selection results of one testing site in contrast to many testing sites, some jatropha genotypes exhibited larger GxE effects than others (Senger *et al.*, 2016a). Therefore, considering data of one testing site resulted in selection of different cultivars than using combined data of several testing sites. The conclusion is that testing at a single location is most risky resulting in selection of genotypes that might be characterized by low yield stability and high response to environmental effects (Senger *et al.*, 2016a). It is therefore of utmost importance for the breeder to evaluate jatropha germplasm at testing sites differing in edapho-climatic conditions. Data of only one or few testing locations, such as in Yi *et al.* (2014), may form part of the germplasm characterization, but are not sufficient to draw conclusions on yield stability.

Stress factors affect morphological and physiological traits and reduce the plants' fitness and yield performance (Hasanuzzaman *et al.*, 2013). Especially physiological parameters are suitable to elucidate short term effects, such as photosynthesis rate, enzyme activity, or respiration. Parameters related to photosynthesis (such as the SPAD value) are of special interest since it is one of the most important processes on Earth and many parameters involved are highly dynamic during the course of a day. A method to record SPAD data in jatropha was developed based on the findings of Nyi *et al.* (2012) to screen numerous plants within short time (Senger *et al.*, 2014a). The SPAD value is strongly correlated with contents of nitrogen and chlorophyll in jatropha leaves, which reflect the physiological status of the plants (Nyi *et al.*, 2012). However, the studies of Nyi *et al.* (2012) were limited to one jatropha genotype, one location, and one time point of data collection. The SPAD method is easier and faster than the commonly used gas exchange measures and it is non-destructive. In our study, a total of 257 genotypes was grown at three locations and screened at different time points within a year. Both, the locations and the time points of measurements, differed significantly in environmental factors such as soil type and precipitation quantity. We found high genetic diversity and significant differences in SPAD value between genotypes, locations, and time points of measurements (Senger *et al.*, 2014a). Since there were only moderate correlations between SPAD value and other traits, this method at the current stage cannot be used to select indirectly for seed yield or yield components. However, it can be

applied for recognition of stress response which will become important in jatropha breeding to identify genotypes with high tolerance to abiotic stresses. For instance, water stress that results in reduced nutrient uptake can be assessed applying the SPAD method. Other environmental stress factors that affect chlorophyll content directly or indirectly can be assessed as well. In a genotype screening regarding aluminum tolerance, SPAD value correctly identified stressed plants that were exposed to aluminum (Senger *et al.*, 2014b).

Aluminum contamination of soils is an abiotic stress factor that occurs in soils with pH value < 5. At low pH, aluminum cations held by clay or humus particles are replaced by free H^+ cations and become diluted in soil water solution. In the tropics and subtropics, acid soils are found mainly in Brazil, African countries near the equator, and South-East Asia. Such soils were originally covered by rain forest or savannah. Large areas have been cleared and transformed into arable land in past decades. Since this is still an ongoing process, it is expected that the area of acid arable land will increase (Uexkuell and Mutert, 1995). Diluted aluminum cations inhibit root growth and lead to oxidative stress of root cells, amongst others (Aggarwal *et al.*, 2015). We developed a rapid screening method that is applied in laboratory scale to identify jatropha genotypes tolerant to aluminum exposure. We found genotypic variation among the tested genotypes and concluded that breeding for improved aluminum tolerance is possible in jatropha (Senger *et al.*, 2014b). However, the results of this study have to be validated at later plant developmental stages and under field conditions. Nevertheless, this method has the potential to be applied with minor modifications to other abiotic stress factors, such as salinity, and enables the breeder to perform efficient germplasm screenings.

Management of jatropha germplasm

Optimal management of germplasm is of utmost importance to assure breeding efficiency. The breeders' germplasm bank needs to be well characterized and a minimum of accessions shall represent the maximum of genetic diversity that is needed to achieve the breeding goals. This prevents redundant genotype testing and saves resources. Based on the characterization, the germplasm shall be grouped depending on objectives and strategies. When breeding

jatropha cultivars for bioenergy and for food production simultaneously, separation of toxic from non-toxic accessions is of utmost importance. The disadvantages of the current PE detection methods are time consuming sample logistics, the need of a laboratory that is in the position to perform the analysis, and extended waiting time in case of high work load of the commissioned laboratory. This is disadvantageous not only for breeders who need fast test results, but also for producers, processors, or traders operating in remote areas, where accessibility of laboratories is limited. The most practical approach would be to develop a rapid portable test kit, for instance based on staining PE or associated compounds similar to the iodine test that is used to prove the presence of starch. However, to the best of my knowledge, such methods or tools have not been published so far for PE detection. On small-scale, bioassays for PE detection were tested (Devappa *et al.*, 2012). However, none of them is at an advanced stage and requires optimization.

Phenotypic markers that are associated to PE presence and that can be assessed with simple methods on plantlets, mature plants, or seeds would be highly desirable. Such phenotypic markers can be quantitative or qualitative traits. The elongated peduncles and oval fruits observed on non-toxic plants by Mulpuri *et al.* (2013) might serve for differentiation of both plant types. However, in quantitative traits, variation within the units of one plant or units of one genotype hinder clear assignment of each unit to the corresponding germplasm pool (Montes *et al.*, 2015; Senger *et al.*, 2016b). The difficulties are random variation and environmental effects that cause data scattering and bias of data, *i.e.*, material growing in one environment cannot be compared to material from another environment. If random variation and environmental effects are large, differentiation between groups is not possible due to overlapping; a fact that many studies (*e.g.* Christo *et al.*, 2014) neglect when calculating significant differences between groups only by using group mean values. Another drawback of the utilization of quantitative traits for group differentiation is the necessity of comparison of the individual under evaluation and the potential genotypes or groups it shall be assigned to. Furthermore, detection of significant differences requires large sample sizes and replicated measurements, which is resource intensive. Therefore, wherever possible, methods based on qualitative traits are preferred.

Promising results for identification of toxic jatropha were obtained in a screening of qualitative seed traits. A trait called “presence of a silver shimmer inside seed testa” was found to differentiate toxic from non-toxic seeds with an error rate of only 3%, which amounted up to 10% for the other traits evaluated (Senger *et al.*, 2015). However, the measured units were not cross-checked, *e.g.*, with molecular markers to confirm the origin from a specific germplasm pool. Thus, it cannot be excluded that the units that did not exhibit the expected phenotype, *i.e.*, caused the error rate, originated from the opposite germplasm pool.

A differentiation based on seed characteristics is only possible during reproductive stages of plant growth or after harvest. It is suitable for testing the harvested material and is therefore of high value for producers, processors, and traders. Promising application areas would be for food security purposes. However, for the breeder, additional traits that can be assessed during vegetative growth stages would be highly desirable. For instance, many non-toxic accessions were reported to have oval fruit shape, while toxic jatropha exhibits round fruit shape (Mulpuri *et al.*, 2013). Vera-Castillo *et al.* (2014) monitored quantitative traits on different non-toxic jatropha accessions and identified eight descriptors (fruit and leaf characteristics) to distinguish between provenances. Both studies show that phenotypic diversity is high within the non-toxic pool and it is difficult to find traits with low variation within the non-toxic pool but high differences between the non-toxic and toxic pools.

Another application of phenotypic markers is the differentiation of self- from cross-fertilized genotypes. Controlled cross-fertilization operations are routinely carried out in cereals, maize, rapeseed, sugar beet, and sunflower by exploiting male sterility or by manual emasculation of floral organs on plants of the seed parent and pollination of the female floral organs with pollen from the pollinator plant. Both methods are resource intensive: Male sterility needs to be introgressed into the seed parent genotype and manual operations are labor-intensive. However, current jatropha seed markets do not encourage high costs for cultivar development and seed production. A third option that is less resource intensive could be to isolate the parental genotypes at flowering. At blossom of the plants, self- and cross-pollination events take place, maybe supported by pollination insects. The harvested seed lot

is then composed of hybrid seed and self-fertilized seeds in varying proportions, depending on the outcrossing rate. To identify and eliminate seeds or plants resulting from self-fertilization, traits that are monitored as early as possible, *i.e.*, on seeds, seedlings, or young plantlets, are needed. In this scenario, the assignment to germplasm pools is not the principal goal, but assignment to the class of hybrid seed or selfed seed. Seeds or seedlings resulting from cross-fertilization shall exhibit a phenotype different to the maternal genotype, *i.e.*, significant paternal or heterotic effects on trait expression are of interest.

An inspiration was the study of Melchinger *et al.* (2013), where maize kernels that resulted from cross-fertilization with a high-oil inducer line could be differentiated from kernels that were not cross-fertilized by kernel oil content. This approach for kernel separation could easily be automated. Assessing quantitative traits on jatropha seeds, Montes *et al.* (2015) found four traits to be affected significantly by the paternal genotype, namely seed oil content, seed mass, kernel mass, and the ratio of kernel to seed mass. Although maternal effects were strong, seeds of specific interpool hybrid combinations could be distinguished from seeds of the maternal genotype (Montes *et al.*, 2015). The effect of the paternal genotype on quantitative seedling traits did not prove to be significant in a study testing similar plant material like in Montes *et al.* (2015). Instead, clear heterotic effects were found in interpool crosses in the characteristics germination time, area of the cotyledon, and area of the primary leaf (Senger *et al.*, 2016b). Out of this list, germination time is probably the most easiest to apply, *e.g.*, in case a maternal genotype of a hybrid is known to exhibit early germination and the paternal genotype late germination, then the seedlings emerging first are discarded. Thinking in large scale seed production, the possibility of automation is highly beneficial. Since seeds are robust to be transported on conveyor belts or screw conveyors and can be separated from the goods flow *e.g.* by compressed air or similar, they are predestined for a mechanized separation system. Automation of separation of seedlings is probably not possible and has to be done manually based on visual observations. On plantations that were established by direct seeding, additional work steps are needed in the field such as removal and resowing or replanting of offtype plants.

Suggestions for jatropha breeding programs

Generation of competitive and widely adapted jatropha cultivars requires a well structured breeding program to achieve highest selection gains and breeding efficiency. The first step might be to generate cultivars with maximized yield potential. This is achieved by combining optimal yield components into one cultivar. Examples in jatropha are early flowering, high number of inflorescences per plant, high number of female flowers per inflorescence, high conversion rate of female flowers to fruits, and high number of seeds per fruit (Islam *et al.*, 2011). To assess the genotypic yield potential, screening of genotypes shall be performed in few testing locations with optimal environmental conditions for jatropha cultivation to reduce the level of stress the plants are exposed to. A description of natural jatropha habitats, a global map of yield estimations based on response to climate factors, and an evaluation of the potential of different soil types for jatropha cultivation are available for site selection (Maes *et al.*, 2009; Trabucco *et al.*, 2010; Jingura *et al.*, 2011). Trait screening needs to be designed as simple as possible by exploiting traits that are easy to monitor and trait associations. The accumulation of favorable traits within the population or into a group of outperforming genotypes can be supported by methods of recurrent selection schemes.

The second step is to secure yield stability, *i.e.*, the plants' capability to realize its yield potential, and consists of resistances/tolerances to biotic/abiotic stress factors. Depending on the target regions for cultivation, the breeder has to define priorities of resistances/tolerances and introgress them into the cultivar with highest yield potential. The superior genotypes selected in the first step need to be tested in multi-factoral field trials that are representative for the targeted cultivation area. One option for genotype screenings for resistances/tolerances to stress factors is monitoring at testing sites, where the stress factors are present or very likely to occur regularly. It requires a broader network of testing sites and more financial resources. To use it as efficient as possible, it shall be reduced to most promising genotypes and important traits. Other options for smart genotype screening shall be exploited, too. For instance, screening for fast juvenile development or resistances and tolerances to specific biotic and abiotic stresses can be performed in greenhouse trials using seedlings or potted plants, if the stress factor(s) can be simulated easily. In comparison to single-location trials,

greenhouse trials bear the additional advantages that environmental factors are standardized and that pest/disease pressure can be homogenized between plants and are not spreading to neighboring plots. However, important interactions between environmental factors as indicated in the study of Al-Busaidi *et al.* (2012) need to be considered while choosing the greenhouse settings. Only the most promising genotypes are then transferred to field testing or to backcrossing programs. It is very likely that heterosis exists in jatropha (Islam *et al.*, 2011; Senger *et al.*, 2016b). Therefore, these two first steps can be supported by exploiting heterotic effects in hybrid breeding to increase yield performance and plant fitness.

In a third step, traits that reduce production costs shall be incorporated into the elite cultivars. The largest fractions of production costs are all manual operations and the fertilizer costs. For instance, dwarf plants are of interest with regard to mechanized harvesting, the necessity of pruning, and planting density; water- and nutrient-efficient cultivars would require less inputs. However, negative associations to yield or plant fitness need to be considered. In the final fourth step, product quality needs to be addressed to provide cultivars with exceptional value to the farmers. However, product quality is defined by the market segment the cultivar is aimed for and by the individual concept of the farmer.

Breeding programs of cultivars for the two market segments are structured differently and the breeder needs to decide on the material to include into the breeding program and on a proper strategy of germplasm management as well. On the one hand, bioenergy production is independent from the presence of phorbol esters, so that this trait can be neglected here. Both toxic and non-toxic germplasm can be used, which bears the advantage of maximized genetic diversity. On the other hand, highly valuable food products can only be produced from seeds of cultivars lacking phorbol esters. For breeding of such non-toxic cultivars, the breeder has two options: In option a), exclusively non-toxic accessions are used, *i.e.* no hybridization with toxic accessions takes place. In option b), the non-toxic and toxic germplasm is mixed and the allele that inhibits phorbol ester synthesis is introgressed into elite material in a (marker-assisted) back crossing scheme. If the breeder chooses option a), the genetic diversity present within the non-toxic pool needs to be sufficient to achieve the breeding objectives. In option b), the advantage of high genetic diversity that leads to high selection gains is counter-

balanced by the disadvantage of the needed backcrossing step. Furthermore, any phenotypic marker used for identification of plants from opposite germplasm pools would not be applicable any longer, except for the original wild germplasm accessions. However, this strategy seems to be consistent with the recommendation of other researchers that renounce the information on phorbol ester presence and suggest to assign jatropha germplasm to potential heterotic groups based on genetic diversity assessed by molecular markers (Biabani *et al.*, 2013; Montes *et al.*, 2014) or phenotypic data (Brasileiro *et al.*, 2013; Sharma *et al.*, 2013).

Tools to identify toxic plants used in breeding programs shall be implemented in commercial plantations and production systems at several points of the process chain. Since molecular markers are published (King *et al.*, 2013), DNA analyses shall be performed for seed lots targeted for sowing, on seedlings in the nursery or in the field before the first harvest, and again on the harvested material. For efficient implementation of molecular markers, analyses of pooled samples are recommendable, which might be supported by phenotypic markers as described above. Especially for the breeder, such phenotypic markers would be highly advantageous. As shown by Montes *et al.* (2015), differentiation of material based on quantitative traits is hindered by large variation between individual units. Therefore, exploiting qualitative traits is more promising. Senger *et al.* (2015) found the trait "silver shimmer inside the seed testa" for differentiation of material of opposite germplasm pools with low error rate. It is a highly interesting trait because it is easy to assess and no laboratory equipment is needed. This trait has high potential to be applied in jatropha breeding programs for efficient germplasm management. However, other phenotypic markers are of interest to identify toxic plants at all stages of plant development. Additionally, the drawbacks of the HPLC method to detect phorbol esters in plant material need to be improved.

Outlook

High genetic diversity and high heritability of important traits has been found in jatropha and will lead to high selection gains. Efficient methods for genotype screening, field testing, and germplasm management were developed. These achievements offer excellent breeding perspectives for jatropha. By selection of promising accessions from the wild germplasm and

combining them into hybrid cultivars, a quantum leap in yield will be achieved. The homozygous nature of most wild accessions (Montes *et al.*, 2014) reduces the time needed to release first generation cultivars because hybridization can be initiated immediately after germplasm characterization.

Proper germplasm management has to be implemented in case cultivars for food and bioenergy production are to be released. The application of efficient analytical tools will support breeding precision and enable breeders to develop cultivars for both market segments at the same time. Additionally, these tools can be adapted by producers, processors, and traders to increase food security. Modern tools and advanced strategies are soon becoming available in jatropha and will further boost breeding progress.

Globally, cultivation of genetically modified cultivars is becoming the standard within intensive agriculture. In jatropha, protocols for *Agrobacterium*-mediated transformation were developed (Mao *et al.*, 2014). Furthermore, particle bombardment, mutagenesis and ecoTILLING have been applied to jatropha as well, but with lower success rate (Joshi *et al.*, 2011; Datta and Pandey, 2013; Maghuly *et al.*, 2013; Dhillon *et al.*, 2014). The major drawbacks of the latter approaches are their rather random action and the unpredictable outcomes. Further technologies are developed continuously such as the CRISPR/Cas or TALEN approaches. It is very likely that genome editing will be applied in jatropha breeding once the cultivation area increases and seed markets demand cultivars with particular monogenic characteristics such as “high-oleic” cultivars or similar.

However, low public acceptance and strict regulations for GMO in the European Union (EU) are limiting trading and utilization of any goods produced from them. It can be expected that the EU legislation will be modified in the near future to cover new techniques as well, such as the CRISPR/Cas or TALEN approaches, which are not covered by the EU legislation currently in force. Therefore, application of any biotechnological technique covered by this legislation is not recommendable if the commercialization of end products is targeted for the EU. Especially the non-toxic kernels are likely to be marketed as food within the EU because their characteristics, as assessed by Senger *et al.* (2017), fit well into current nutritional trends in Europe, such as vegan or “low-carb” diets.

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X Summary

Jatropha curcas L. (jatropha) is a perennial plant of the Euphorbiaceae family that grows in the tropics and subtropics worldwide. Jatropha is targeted to be grown in marginal environments. The seeds are used mainly for production of food products and bioenergy, amongst others. Jatropha breeding is at an early stage. The first obstacle is to generate competitive cultivars for economically feasible cultivation. Major breeding objectives are to increase seed yield and yield stability, to decrease production costs, and to improve product quality adapted to specific markets. Jatropha breeding needs to be optimized in several research areas, such as methods and tools for germplasm characterization and breeding techniques, while considering requirements of the agronomic management and product processing.

The germplasm can be separated into two naturally occurring germplasm pools that differ in the presence of phorbol esters (PE). These chemical compounds have antinutritional effects on humans and animals and cannot be inactivated or eliminated from the plant material on an industrial scale yet. Therefore, food production is based on cultivars lacking PE, while bioenergy production is less affected from PE presence. The germplasm needs to be characterized and grouped depending on breeding objectives and strategies. Tools for identification of plants that synthesize PE exist, but bear decisive disadvantages or need to be advanced. These tools are exploited for germplasm management and food safety strategies.

The objectives of this study were to i) examine the variation of relevant traits among genotypes and between germplasm pools, ii) estimate phenotypic and genotypic trait correlations, iii) investigate location effects and genotype by environment interactions, iv) investigate parental and heterotic effects of genotypes from different germplasm pools as well as the effect of the mating type on expression of relevant traits, and v) develop recommendations for implementation of the findings in jatropha breeding programs.

In the first two publications, stress response was investigated. Leaf chlorophyll content (SPAD) was used as a dynamic trait that can be influenced by *e.g.* water stress and nutrient deficiency. Different genotypes were screened at several locations and at different time points. High genetic diversity was found not only in stress response but also in SPAD value. The fast and non-destructive method is highly promising to be applied in further screenings or

stress response studies. In the second publication, genotypic differences in aluminum tolerance were found among seedlings in a greenhouse trial. The rapid test method is applicable in further screenings. However, it needs to be proven that aluminum tolerance at the seedling stage observed under greenhouse conditions is expressed also at later plant developmental stages in the field.

In the consecutive three publications, several traits were assessed on seeds and seedlings to detect significant differences between genotypes and/or between germplasm pools. Such traits would be highly valuable for germplasm management. We found that random variation is a disadvantage of quantitative traits and hinders clear assignment of each experimental unit to the respective germplasm pool. Thus, qualitative traits might be favored, such as the "silver shimmer inside the seed testa" that differentiated toxic from non-toxic seeds with a low error rate. However, these results need to be validated. Another application area of the investigated traits is the identification of self-fertilized material within hybrid progeny. In our study, self-fertilized seeds could be differentiated from cross-fertilized ones in specific genotype combinations. Similarly, many seedling traits showed heterotic effects.

In the sixth publication, genotype by environment interactions were investigated and recommendations for breeding programs elaborated. A large set of genotypes was grown for four years at three different locations. We showed that selection at only one testing location is highly risky because cultivars with low yield stability could be selected. Therefore, it is indispensable for breeders to work in a network of testing locations that differ in edapho-climatic conditions and apply appropriate experimental designs and statistical tools.

In the final publication, several parameters related to the nutritional value of kernels of non-toxic genotypes grown at two locations were assessed. The high nutritional value of this material was presented and compared to soybean, peanut, hazelnut, and corn. Furthermore, preliminary conclusions related to location effects and product processing were drawn.

The findings of this thesis contribute to characterization of this novel crop with regard to stress tolerances, seed and seedling characteristics as well as food quality, and help to increase breeding efficiency by presenting simple methods for fast genotype screening as well as grouping of germplasm and by efficient exploitation of testing facilities.

XI Zusammenfassung

Jatropha curcas L. (Jatropha) ist eine perennierende Pflanze der Familie der Euphorbiaceen mit weltweiter Verbreitung in den Tropen und Subtropen und eignet sich für den Anbau in marginalen Umwelten. Die Samen werden u.a. in der Bioenergie- und Lebensmittelproduktion genutzt. Die Züchtung dieser Pflanzenart befindet sich noch in den Anfängen. Zunächst müssen Sorten generiert werden, die eine profitable Produktion ermöglichen. Die Zuchtziele liegen primär in der Steigerung von Ertrag und Ertragsstabilität sowie der Senkung der Produktionskosten, sekundär in der Verbesserung der Produktqualität in Abhängigkeit der Ansprüche des jeweiligen Absatzmarktes. Jatrophazüchtung muss in mehreren Bereichen optimiert werden, insbesondere in der Entwicklung von Methoden zur Charakterisierung des Materials, sowie geeigneten Züchtungsverfahren, wobei Anforderungen seitens des agronomischen Managements und der Produktverarbeitung zu berücksichtigen sind.

Jatropha kann in zwei natürlich vorkommende Genpools unterteilt werden, die sich im Vorhandensein von sogenannten Phorbolestern (PE) unterscheiden. Diese chemischen Substanzen haben antinutritive Effekte auf Mensch und Tier und können noch nicht in industriellem Maßstab entfernt oder neutralisiert werden. Daher basiert die Lebensmittelproduktion aus Jatropha auf Sorten, die keine PE enthalten, während Bioenergie unabhängig vom Gehalt an PE produziert werden kann. Das Material muss in Abhängigkeit von Zuchtzielen und -strategien charakterisiert und gruppiert werden. Es gibt Methoden zur Identifizierung von PE synthetisierenden Pflanzen. Diese haben jedoch entscheidende Nachteile oder müssen weiter optimiert werden. Diese Methoden werden in der Züchtung und zur Überwachung der Lebensmittelreinheit genutzt.

Die Ziele der vorliegenden Dissertation waren i) die Quantifizierung von Merkmalsvariationen zwischen Genotypen und Genpools, ii) die Bestimmung von phänotypischen und genotypischen Merkmalskorrelationen, iii) die Untersuchung von Standorteffekten und Genotyp-Umwelt-Interaktionen, iv) die Analyse sowohl von elterlichen und heterotischen Effekten als auch von Bestäubungseffekten in Genotypen verschiedener Genpools, sowie v) die Erarbeitung von Empfehlungen für Jatropha-Zuchtprogramme zur Umsetzung der Untersuchungsergebnisse.

In den ersten zwei Publikationen wurde die Reaktion auf Stressfaktoren untersucht. Der Chlorophyllgehalt in Blättern (SPAD) wurde als dynamisches Merkmal verwendet, um die Stressreaktion zu erfassen. An drei Standorten wurde der SPAD-Wert verschiedener Genotypen zu mehreren Zeitpunkten gemessen. Wir stellten eine hohe genetische Diversität fest, nicht nur im Ausmaß der Stressreaktion, sondern auch im SPAD-Wert. Die schnelle und nicht-destruktive Methode eignet sich bestens für weitere Screenings oder Studien zu Stressreaktionen. In der zweiten Publikation wurden genotypische Unterschiede in der Aluminiumtoleranz von Keimlingen in einem Gewächshausversuch festgestellt. Der Schnelltest kann gut in Screenings angewendet werden. Allerdings muss nachgewiesen werden, dass die Aluminiumtoleranz auch zu späteren Entwicklungsstadien der Pflanze besteht.

In den darauf folgenden drei Publikationen wurden quantitative und qualitative Merkmale an Samen und Keimlingen erfasst, um evtl. vorhandene Unterschiede zwischen Genpools oder Genotypen zu ermitteln. Solche Merkmale wären für die Materialgruppierung sehr wertvoll. Die hier untersuchten quantitativen Merkmale hatten den Nachteil nicht-genetischer Variation, die keine eindeutige Klassifizierung erlaubte. Daher könnten qualitative Merkmale vielversprechender sein, wie z.B. der „silberne Glanz auf der Innenseite der Samentesta“, mit dessen Hilfe es möglich war, toxische von nicht-toxischen Samen mit einer geringen Fehlerrate zu unterscheiden. Allerdings müssen diese Ergebnisse noch validiert werden. Ein weiterer Anwendungsbereich von quantitativen und qualitativen Merkmalen ist die Trennung von (vermischten) Selbstungs- und Hybridgenotypen. In dieser Untersuchung konnten selbstbestäubte Samen in bestimmten Hybridkombinationen erkannt werden. Ebenso zeigten viele an Keimlingen erfasste Merkmale heterotische Effekte.

In der sechsten Publikation wurden Genotyp-Umwelt-Interaktionen untersucht und Empfehlungen für Züchtungsprogramme erarbeitet. Zahlreiche Genotypen wurden über vier Jahre an drei Standorten beobachtet. Es wurde gezeigt, dass die Selektion auf Basis einortiger Ergebnisse sehr risikoreich ist, da Genotypen mit geringer Ertragsstabilität selektiert werden könnten. Daher ist es für Züchter unumgänglich mit einem Netzwerk von edapho-klimatisch unterschiedlichen Prüfstandorten zu arbeiten.

In der finalen Publikation wurden ernährungsphysiologische Merkmale an Kernen von nicht-toxischen Genotypen aus zwei Standorten erfasst. Es ergab sich ein hoher ernährungsphysiologischer Wert im Vergleich zu Soja, Erdnuss, Haselnuss und Mais. Außerdem konnten Schlussfolgerungen bezüglich Standorteffekten und Produktverarbeitung gezogen werden.

Die Ergebnisse der vorliegenden Arbeit tragen zur Charakterisierung dieser neuen Kulturpflanze in Hinblick auf Stresstoleranzen, Samen- und Keimlingsmerkmale und Lebensmittelqualität bei. Sie sind daher für eine Steigerung der Zuchteffizienz hilfreich, indem sowohl einfache Methoden für schnelle Screenings und für die Materialgruppierung vorgestellt werden, als auch die effiziente Ausnutzung von Prüfressourcen diskutiert wird.

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